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(71) Applicant: MEDICAL RESEARCH COUNCIL [GB/GB]; 20 Park Crescent, London W1B 1AL (GB).

(72) Inventor: JABBOUR, Henry, Nicolas; MRC Human Reproductive Sciences Unit, 37 Chalmers Street, Edinburgh EH3 9ET (GB).

(74) Agent: MILES, John, S; Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 5DD (GB).

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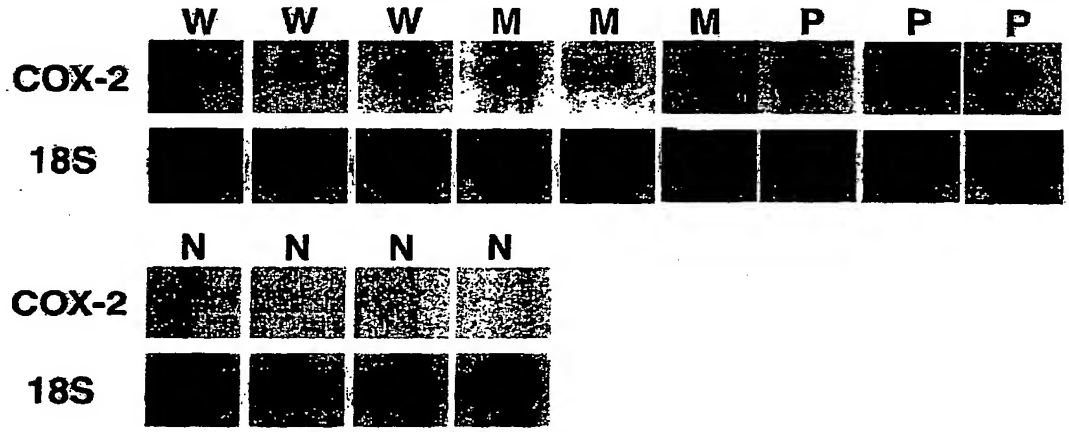

(57) Abstract: A method of treating or preventing a pathological condition of the uterus in an individual the method comprising administering to the individual any one or more of an inhibitor of prostaglandin E synthase (PGES), or an EP2 OR EP4 receptor antagonist. Optionally, the patient is also administered an inhibitor of COX-2. Typically, the pathological condition is uterine cancer, fibroids or endometriosis.

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USE OF PROSTAGLANDIN E SYNTHASE INHIBITORS, OR EP2 OR EP4 RECEPTOR ANTAGONISTS, IN THE TREATMENT OF A PATHOLOGICAL CONDITION OF THE UTERUS

The present invention relates to methods of treatment, and in particular methods of treating uterine pathological conditions.

Pathological conditions of the uterus represent a serious health problem in women, particularly women of the Western world. Such pathological conditions include uterine carcinoma, and endometrial or myometrial pathological conditions such as endometriosis (endometrial) and fibroids (myometrial).

Cyclooxygenase (COX) enzymes, also called prostaglandin endoperoxide synthase, (PGHS), catalyse the rate limiting step in the conversion of arachidonic acid to prostaglandin H_2 (PGH_2). In turn PGH_2 serves as a substrate for specific prostaglandin synthase enzymes that synthesise the natural prostaglandins. These are named according to the prostaglandin they produce such that prostaglandin D_2 is synthesised by prostaglandin-D-synthase, prostaglandin E_2 (PGE_2) by prostaglandin-E-synthase (PGES) and prostaglandin $F_{2\alpha}$ by prostaglandin-F-synthase. To-date, there are two identified isoforms of the COX enzyme, COX-1 and COX-2 (DeWitt, 1991). COX-1 is constitutively expressed in many tissues and cell types and generates prostaglandins for normal physiological function (Herschman, 1996). By contrast, the expression of COX-2 is rapidly induced following stimulation of quiescent cells by growth factors, oncogenes, carcinogens and tumour-promoting phorbol esters (Herschman, 1996; Subbaramaiah *et al.*, 1996). In addition, two isoforms of PGES have been isolated; a microsomal glutathione-dependent inducible PGES (mPGES) and a constitutive cytosolic glutathione dependent PGES (Jakobsson *et al.*, 1999; Tanioka *et al.*, 2000). *In vitro* studies support the idea that COX-2 and possibly PGE_2 are involved in neoplastic

transformation of certain epithelial cells and subsequently carcinogenesis. Over-expression of COX-2 and PGE_2 synthesis in rat intestinal epithelial cells increases their proliferation rate, resistance to apoptosis, and their invasiveness by suppressing the transcription of target genes that may be involved in cellular growth/transformation and adhesion (Tsujii & DuBois, 1995). In addition, it has been proposed recently that COX-2 and PGE_2 promote cancer development and invasiveness by mediating the transcription of angiogenic factors that induce both migration of endothelial cells and their arrangement into tubular structures (Tsujii *et al.*, 1998; Jones *et al.*, 1999b).

Cyclooxygenases have been studied in various cancers, but the picture that has emerged to date is confusing and it is not possible to predict the role of COX-1 or COX-2 in any particular cancer. For example, both COX-1 and COX-2 have been shown to be highly expressed in lung cancer in the mouse (Bauer *et al* (2000) *Carcinogenesis* **21**, 543-550) whereas Rioux & Castonguay (2000) *Carcinogenesis* **21**, 1745-1751 indicates that COX-1 is induced by tobacco carcinogens in human macrophages and is correlated with NFκB activation. According to Doré *et al* (1998) *J. Histochem. Cytochem.* **46**, 77-84 COX-1 but not COX-2 is expressed in human ovarian adenocarcinomas. According to Ryu *et al* (2000) *Gynecologic Oncology* **76**, 320-325 COX-2 expression is high in stage IB cervical cancer whereas COX-1 was expressed without regard to location of the tumour cells or type of cancer cell and the authors indicate that COX-1 is unrelated to apoptosis, tumourigenesis and tumour invasion mechanisms. Kulkarni *et al* (2001) *Clin. Cancer Res.* **7**, 429-434 indicates that COX-2 is overexpressed in human cervical cancer.

PGE_2 mediates its effect on target cells through interaction with different isoforms of seven transmembrane G protein coupled receptors (GPCR)

which belong to the rhodopsin family of serpentine receptors. Four main PGE_2 receptor subtypes have been identified (EP1, EP2, EP3 and EP4) which utilise alternate and in some cases opposing intracellular signalling pathways (Coleman *et al.*, 1994). This diversity of receptors with opposing action may confer a homeostatic control on the action of PGE_2 that is released in high concentrations close to its site of synthesis (Ashby, 1998). To-date, the role of the different PGE_2 receptors, their divergent intracellular signalling pathways, as well as their respective target genes involved in mediating the effects of PGE_2 on normal or neoplastically transformed endometrial epithelial cells remain to be elucidated.

Epithelial cells of the human endometrium are highly vulnerable to neoplastic transformation. In the western world, endometrial carcinoma is the most common gynaecologic malignancy. Endometrial cancer can arise from several cell types but the glandular epithelium is the most common progenitor (adenocarcinomas account for 80-90% of uterine tumours). Endometrial cancer is predominantly a post-menopausal disease where incidence is uncommon below the age of forty and peaks by about seventy years of age. The incidence of endometrial cancer has been increasing steadily in the Western world during the last fifty years and this has been attributed largely to increased life expectancy and improved detection methods (Gordon & Ireland, 1994; Mant & Vessey, 1994).

We found in the study described in Example 1 that COX-2 and, surprisingly, mPGES expression and PGE_2 synthesis are up-regulated in adenocarcinoma of the human uterus. Expression of these factors was localised to the neoplastic epithelial cells of the uterine carcinoma tissues as well as the endothelial cells of the microvasculature. This is associated with an overexpression, and signalling of, the EP2 and EP4 receptors in the carcinoma tissue.

A first aspect of the invention provides a method of treating a pathological condition of the uterus in an individual the method comprising administering to the individual any one or more of an inhibitor of prostaglandin E synthase (PGES), or an EP2 or EP4 receptor antagonist.

The pathological condition of the uterus may be any pathological condition wherein synthesis of PGE2 is found, and wherein expression of EP2 and EP4 receptors is found. Typically the pathological condition of the uterus is any one of uterine carcinoma, an endometrial pathological condition such as endometriosis including adenomyosis, or a myometrial pathological condition such as fibroids (leiomyomas) or leiomyosarcomas which are fibroids which have become malignant. Thus, typically, the uterine pathological condition is one which is associated with abnormal growth of cells of the myometrium or endometrium. Endometriosis is the ectopic implantation and growth of endometrium and can therefore be considered as abnormal growth cells of the endometrium as defined. Adenomyosis is a form of endometriosis where the ectopic endometrium is implanted in the myometrium.

It is particularly preferred if the method of the invention is used to treat endometrial carcinoma.

In one embodiment of the invention, the individual is administered an inhibitor of PGES. It has been reported by Thorén & Jakobsson (2000) *Eur. J Biochem.* **267**, 6428-6434 (incorporated herein by reference) that NS-398, sulindac sulphide and leukotriene C_4 inhibit PGES activity with IC_{50} values of 20μM, 80μM and 5μM, respectively.

In a further embodiment of the invention, the individual is administered an antagonist of an EP2 receptor or an antagonist of an EP4 receptor.

The prostaglandin EP2 receptor antagonist may be any suitable EP2 receptor antagonist. Similarly, the prostaglandin EP4 receptor antagonist may be any suitable EP4 receptor antagonist. By "suitable" we mean that the antagonist is one which may be administered to the patient. The receptor antagonists are molecules which bind to their respective receptors, compete with the natural ligand (PGE_2) and inhibit the initiation of the specific receptor-mediated signal transduction pathways. The receptor antagonists are typically selective to the particular receptor and typically have a higher binding affinity to the receptor than the natural ligand. Although antagonists with a higher affinity for the receptor than the natural ligand are preferred, antagonists with a lower affinity may also be used, but it may be necessary to use these at higher concentrations. Preferably, the antagonists bind reversibly to their cognate receptor. Typically, antagonists are selective for a particular receptor and do not affect the other receptor; thus, typically, an EP2 receptor antagonist binds the EP2 receptor but does not substantially bind the EP4 receptor, whereas an EP4 receptor antagonist binds the EP4 receptor but does not substantially bind the EP2 receptor. Preferably, the EP2 or EP4 receptor antagonist is selective for the particular receptor subtype. By this is meant that the antagonist has a binding affinity for the particular receptor subtype which is at least ten-fold higher than for at least one of the other EP receptor subtypes. Thus, selective EP4 receptor antagonists have at least a ten-fold higher affinity for the EP4 receptor than any of the EP1, EP2 or EP3 receptor subtypes.

It is particularly preferred that the EP2 or EP4 receptor antagonist is selective for its cognate receptor.

The EP2 or EP4 receptor antagonists are typically administered in an effective amount to combat the pathological condition of the uterus. Thus, the antagonists may be used to alleviate symptoms (ie are used palliatively) or may be used to treat the condition. The antagonist may be administered prophylactically (and by "treating" we include prophylactic treatment). The antagonist may be administered by any suitable route, and in any suitable form. It is desirable to administer an amount of the EP2 or EP4 receptor antagonist that is effective in preventing or alleviating or ameliorating or curing the pathological condition of the uterus.

EP2 receptor antagonists include AH6809 (Pelletier *et al* (2001) *Br. J. Pharmacol.* **132**, 999-1008).

EP4 receptor antagonists include AH23848B (developed by Glaxo) and AH22921X (Pelletier *et al* (2001) *Br. J. Pharmacol.* **132**, 999-1008. The chemical name for AH23848B is ([1alpha(z), 2beta5alpha]-(+/-)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-2-(4-morph olinyl)-3-oxo-cyclopentyl]-4-heptenoic acid) (see Hillock & Crankshaw (1999) *Eur. J. Pharmacol.* **28**, 99-108). EP4RA (Li (2000) Endocrinology **141**, 2054-61) is an EP(4) - selective ligand (Machwate *et al* (2001) *Mol. Pharmacol.* **60**: 36-41). The omega-substituted prostaglandin E derivatives described in WO 00/15608 (EP 1 114 816) (Ono Pharm Co Ltd) bind EP4 receptors selectively and may be EP4 receptor antagonists.

Peptides described in WO 01/42281 (Hopital Sainte-Justine) eg: IFTSYLECL, IFASYECL, IFTSAECL, IFTSYEAL, ILASYECL, IFTSTDCL, TSYEAL (with 4-biphenyl alanine), TSYEAL (with homophenyl alanine) are also described as EP4 receptor antagonists, as are some of the compounds described in WO 00/18744 (Fujisawa Pharm Co

Ltd). The 5-thia-prostaglandin E derivatives described in WO 00/03980 (EP 1 097 922) (Ono Pharm Co Ltd) may be EP4 receptor antagonists.

EP4 receptor antagonists are also described in WO 01/10426 (Glaxo), WO 00/21532 (Merck) and GB 2 330 307 (Glaxo).

WO 00/21532 describes the following as EP4 receptor antagonists:

5-butyl-2,4-dihydro-4-[[2'-[N-(3-chloro-2-thiophenecarbonyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one potassium salt;

5-butyl-2,4-dihydro-4-[[2'-[N-(2-methyl-3-furoyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one;

5-butyl-2,4-dihydro-4-[[2'-[N-(3-methyl-2-thiophenecarbonyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one;

5-butyl-2,4-dihydro-4-[[2'-[N-(2-thiophenecarbonyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one;

5-butyl-2,4-dihydro-4-[[2'-[N-[2-(methypyrrole)carbonyl]sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one.

GB 2 330 307 describes [1α(Z), 2β,5α]-(±)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid and [1R[1α(z),2β,5α]]-(-)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid.

WO 00/18405 (Pharmagene) describes the EP4 receptor antagonists AH22921 and AH23848 (which are also described in GB 2 028 805 and US 4, 342, 756). WO 01/72302 (Pharmagene) describes further EP4 receptor antagonists, for example those described by reference to, and included in the general formula (I) shown on page 8 *et seq*.

All of these references to EP2 and EP4 receptor antagonists are incorporated herein by reference.

It will be appreciated that one or more EP2 receptor antagonists, or one or more EP4 receptor antagonists, may be administered to the patient. It will also be appreciated that a combination of one or more EP2 or EP4 receptor antagonists may be administered to the patient.

In a further embodiment of the invention, the individual is additionally administered a COX-2 inhibitor. Preferably the inhibitor is selective for COX-2.

The compound may selectively inhibit COX-2 function at any level. Suitably, the compound selectively inhibits COX-2 enzyme activity.

By "selectively inhibits COX-2 enzyme activity" we mean that the compound preferably inhibits COX-2 in preference to other cyclo-oxygenase enzymes, in particular in preference to cyclo-oxygenase-1 (COX-1). The COX-1 gene and the sequence of its polypeptide product are described in Yokoyama and Tanabe (1989) *Biochem. Biophys. Res. Comm.* **165**, 888-894 incorporated herein by reference. COX-1 is also called PGHS-1. The COX-2 gene and the sequence of its polypeptide product are

described in O'Banion *et al* (1991) *J Biol. Chem.* **266,** 23261-23267 incorporated herein by reference. COX-2 is also called PGHS-2.

Conveniently, the compound which selectively inhibits COX-2 enzyme activity is at least ten times better at inhibiting COX-2 than COX-1; preferably it is at least fifty times better; preferably it is at least one hundred times better; still more preferably it is at least one thousand times better and in greater preference it is at least ten thousand times better.

It is most preferred if the compound has substantially no inhibitory activity against the COX-1 enzyme.

Conveniently, the compound selectively inhibits COX-2 enzyme production. The compound may, for example, selectively prevent transcription of the COX-2 or it may selectively prevent translation of the COX-2 message.

By "selectively inhibits COX-2 enzyme production" we mean that the compound preferably inhibits the production of COX-2 in preference to other cyclo-oxygenases, in particular in preference to the production of COX-1.

Conveniently, the compound which selectively inhibits COX-2 enzyme production is at least ten times better at inhibiting COX-2 production than COX-1 production; preferably it is at least fifty times better; more preferably it is at least one hundred times better; more preferably still it is at least one thousand times better; and in greater preference it is at least ten thousand times better.

It is most preferred if the compound has substantially no inhibitory activity against COX-1 enzyme production.

A particularly preferred embodiment is wherein the compound is any one of nimesulide, 4-hydroxynimesulide, flosulide, and meloxicam.

Nimesulide is N-(4-nitro-2-phenoxyphenyl) methanesulfonamide (also called 4-nitro-2-phenoxymethanesulfonanilide). Nimesulide is 100-fold more specific for COX-2 inhibition than for COX-1 inhibition. Nimesulide is manufactured by Boehringer.

Flosulide is 6-(2,4-difluorophenoxy)-5-methyl sulphonylamino-1-indanone (also known as N-6-(2,4-difluorophenoxy)-1-oxo-indan-5-yl methane-sulphonamide). Flosulide is 1000-fold more specific for COX-2 inhibition than for COX-1 inhibition. Flosulide is manufactured by Ciba Geigy.

Meloxicam is 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide. Meloxicam is 1000-fold more specific for COX-2 inhibition than for COX-1 inhibition. Meloxicam is manufactured by Boehringer.

The synthesis of nimesulide is well known and is described in US 3,840,597; the synthesis of flosulide is well known and is described in GB 2 092 144; and the synthesis of meloxicam is well known and is described in US 4,233,299.

Other COX-2-specific inhibitors which may be useful in the practice of the invention include:

L 475 L337 which is 500-fold more specific for COX-2 inhibition than for COX-1 inhibition. This is manufactured by Merck Frost.

Vioxx, sold by Merck, is also a suitable COX-2 inhibitor.

SC 58125 Celecoxib which is 100-fold more specific for COX-2 inhibition than for COX-1 inhibition. Celecoxib is manufactured by Searle.

NS 398 which is manufactured by Taisho and which is very highly selective for COX-2.

DuP 697, which is COX-2-selective and is manufactured by DuPont.

Nimesulide, flosulide and meloxicam are COX-2 enzyme inhibitors, probably competitive inhibitors.

It may be advantageous to administer to the individual a combination of one or more of the PGES inhibitor or antagonist of EP2 receptor or antagonist of EP4 receptor and, optionally, one or more COX-2 inhibitors. These may all be considered "treatment agents" of the invention.

The treatment agents are administered in an effective amount to combat the undesired pathological condition of the uterus. Thus, the treatment agents may be used to alleviate symptoms (ie are used palliatively) or may be used to treat the condition or may be used prophylactically to prevent the condition. The treatment agent may be administered by any suitable route, and in any suitable form. The aforementioned treatment agents for use in the invention or a formulation thereof may be administered by any conventional method including oral and parenteral (eg subcutaneous or intramuscular) injection. The treatment may consist of a single dose or a

plurality of doses over a period of time. The dose to be administered is determined upon consideration of age, body weight, mode of administration, duration of the treatment and pharmacokinetic and toxicological properties of the treatment agent or agents. The treatment agents are administered at a dose (or in multiple doses) which produces a beneficial therapeutic effect in the patient. Typically, the treatment agents are administered at a dose the same as or similar to that used when the treatment agent is used for another medical indication. In any event, the dose suitable for treatment of a patient may be determined by the physician.

Whilst it is possible for a treatment agent of the invention to be administered alone or in combination with other said treatment agents, it is preferable to present it or them as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the treatment agent of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the treatment agent or agents with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient (ie treatment agent or agents) with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a

powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (eg sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier. Buccal administration is also preferred.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions

which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

Certain EP2 and EP4 receptor antagonists are proteins or peptides. Proteins and peptides may be delivered using an injectable sustained-release drug delivery system. These are designed specifically to reduce the frequency of injections. An example of such a system is Nutropin Depot which encapsulates recombinant human growth hormone (rhGH) in biodegradable microspheres that, once injected, release rhGH slowly over a sustained period.

The protein and peptide can be administered by a surgically implanted device that releases the drug directly to the required site. For example, Vitrasert releases ganciclovir directly into the eye to treat CMV retinitis. The direct application of this toxic agent to the site of disease achieves effective therapy without the drug's significant systemic side-effects.

Electroporation therapy (EPT) systems can also be employed for the administration of proteins and peptides. A device which delivers a pulsed electric field to cells increases the permeability of the cell membranes to the drug, resulting in a significant enhancement of intracellular drug delivery.

Proteins and peptides can be delivered by electroincorporation (EI). EI occurs when small particles of up to 30 microns in diameter on the surface of the skin experience electrical pulses identical or similar to those used in electroporation. In EI, these particles are driven through the stratum corneum and into deeper layers of the skin. The particles can be loaded or coated with drugs or genes or can simply act as "bullets" that generate pores in the skin through which the drugs can enter.

An alternative method of protein and peptide delivery is the ReGel injectable system that is thermo-sensitive. Below body temperature, ReGel is an injectable liquid while at body temperature it immediately forms a gel reservoir that slowly erodes and dissolves into known, safe, biodegradable polymers. The EP2 or EP4 receptor antagonist is delivered over time as the biopolymers dissolve.

Protein and peptide pharmaceuticals can also be delivered orally. The process employs a natural process for oral uptake of vitamin B_{12} in the body to co-deliver proteins and peptides. By riding the vitamin B_{12} uptake system, the protein or peptide can move through the intestinal wall. Complexes are synthesised between vitamin B_{12} analogues and the drug that retain both significant affinity for intrinsic factor (IF) in the vitamin B_{12} portion of the complex and significant bioactivity of the drug portion of the complex.

Proteins and polypeptides can be introduced to cells by "Trojan peptides". These are a class of polypeptides called penetratins which have translocating properties and are capable of carrying hydrophilic compounds across the plasma membrane. This system allows direct targetting of oligopeptides to the cytoplasm and nucleus, and may be non-cell type specific and highly efficient. See Derossi *et al* (1998), *Trends Cell Biol* **8**, 84-87.

It is preferred if the treatment agent (or agents) is administered orally. It is further preferred if the treatment agent (or agents) is administered to the female reproductive system. For example, the treatment agent or agents may suitably be administered intravaginally using, for example, a gel or cream or vaginal ring or tampon. The treatment agent may also advantageously be administered by intrauterine delivery, for example using methods well known in the art such as an intrauterine device.

Typically, the gel or cream is one which is formulated for administration to the vagina. It may be oil based or water based. Typically, the treatment agent (or agents) is present in the cream or gel in a sufficient concentration so that an effective amount is administered in a single (or in repeated) application.

Typically, the vaginal ring comprises a polymer which formed into a "doughnut" shape which fits within the vagina. The treatment agent (or agents) is present within the polymer, typically as a core, which may dissipate through the polymer and into the vagina and/or cervix in a controlled fashion. Vaginal rings are known in the art.

Typically, the tampon is impregnated with the treatment agent (or agents) and that a sufficient amount of the treatment agent (or agents) is present in the tampon.

Typically, the intrauterine device is for placing in the uterus over extended periods of time, such as between one and five years. Typically, the intrauterine device comprises a plastic frame, often in the shape of a "T" and contains sufficient antagonist to be released over the period of use. The antagonist is generally present within or encompassed by a slow-release polymer which forms part of the device, such as in the form of a "sausage" of antagonist which wraps around the long arm of the "T" which is typically covered with a controlled-release membrane. Intrauterine devices are known in the art.

The individual to be treated may be any female individual who would benefit from such treatment. Typically and preferably the individual to be treated is a human female. However, the methods of the invention may be used to treat female mammals, such as the females of the following species: cows, horses, pigs, sheep, cats and dogs. Thus, the methods have uses in both human and veterinary medicine.

A second aspect of the invention provides use of any one or more of an inhibitor of prostaglandin E synthase (PGES), or an EP2 or EP4 receptor antagonist in the manufacture of a medicament for treating or preventing a pathological condition of the uterus.

The medicament may usefully further comprise an inhibitor of COX-2.

In a further embodiment the medicament is for administration to a patient who is administered a COX-2 inhibitor and/or an inhibitor of PGES and/or

an EP2 or EP4 receptor antagonist. Thus, the patient may have been administered one of the treatment agent or agents previously, or is administered them simultaneously or is administered them after the treatment agent or agents present in the medicament.

The invention will now be described in more detail by reference to the following Examples and Figures wherein:

Figure 1. Ribonuclease protection assay conducted using 10 μg of total RNA extracted from normal secretory phase endometrium (N) and well (W), moderately (M) and poorly (P) differentiated endometrial adenocarcinoma tissue. COX-2 expression was detected using a 381 homologous cRNA probe. The integrity of the RNA and the relative amount of total RNA in each reaction were determined using a ribosomal 18S cDNA probe.

Figure 2. COX-2 expression and PGE_2 synthesis are detected in epithelial cells of poorly (Figure a and b respectively), moderately (Figure c and d respectively) and well (Figure e and f respectively) differentiated endometrial adenocarcinoma. Minimal immunostaining for COX2 or PGE_2 were detected in post menopausal (Figure g and h) or secretory phase (Figure i and j) endometrium. Insets in Figure 2e and 2f are sections that were stained with pre-adsorbed COX-2 and PGE_2 sera respectively (negative controls). Scale bar is 100 μm.

Figure 3. mPGES expression is detected in epithelial cells of poorly (Figure a), moderately (Figure b) and well (Figure c) differentiated endometrial adenocarcinoma. Minimal immunostaining for mPGES was detected in post-menopausal uterus and secretory phase endometrium (Figure d and e respectively). Inset in Figure c is a section that was stained with mPGES pre-adsorbed serum (negative control). Scale bar is 100 μm.

Figure 4. COX-2 (Figure a), mPGES (Figure b), PGE_2 (Figure c) and EP4 (Figure d) are detected in endothelial cells of all carcinoma tissues. Vascular endothelial cells in endometrial adenocarcinoma were localised using antibodies raised against the human CD34 endothelial cell marker (Figure e). The inset in Figure e is a section that was stained with non-immune goat serum (CD34 negative control). Negative controls for the other antibodies are presented in Figures 2, 3 and 5B. Scale bar is 50 μm.

Figure 5. A. Relative expression of EP2 and EP4 receptors in endometrial adenocarcinoma of different grades of differentiation and in healthy secretory phase endometrium collected from fertile women with normal menstrual cycles. **B.** EP4 receptor expression was detected in neoplastic epithelial cells of poorly (Figure a), moderately (Figure b) and well (Figure c) differentiated uterine adenocarcinomas. The inset in Figure c is a section that was stained with immune serum that had been pre-adsorbed with the blocking peptide. Scale bar is 50 μm.

Figure 6. Fold induction of cAMP response in endometrial adenocarcinoma (n=6) and healthy secretory phase endometrium (n=6) following stimulation with 300 nM PGE_2. Fold induction was calculated by dividing cAMP values for the PGE_2-treated samples by the values for the untreated sample.

Example 1: Expression of COX-2 and PGE synthase and synthesis of PGE_2 in endometrial adenocarcinoma: a possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors

Summary

This study was designed to investigate the possible role of cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) in endometrial adenocarcinoma.

COX-2 RNA expression was confirmed in various grades of adenocarcinoma by ribonuclease protection assay. COX-2 and microsomal glutathione-dependent prostaglandin E synthase (mPGES) expression and PGE_2 synthesis were localised to the neoplastic epithelial cells and endothelial cells. In order to establish whether PGE_2 has an autocrine/paracrine effect in adenocarcinomas, we investigated the expression of two subtypes of PGE_2 receptors, namely EP2 and EP4, by real time quantitative PCR. Expression of EP2 and EP4 receptors was detected in adenocarcinomas from all grades of differentiation and was significantly higher than that detected in normal secretory phase endometrium ($P<0.01$). The fold induction of expression in adenocarcinoma compared with normal secretory phase endometrium was 28.0 ± 7.4 and 52.5 ± 10.1 for EP2 and EP4 receptors respectively. Immunohistochemistry localised the site of expression of EP4 receptor in neoplastic epithelial cells and in the endothelium of carcinomas of all grades of differentiation. Finally, the functionality of the EP2/EP4 receptors was assessed by investigating cAMP generation following in vitro culture of adenocarcinoma tissue in the presence or absence of 300nM PGE_2. cAMP production in response to PGE_2 was significantly higher in carcinoma tissue than that detected in normal secretory phase endometrium (3.42 ± 0.46 vs 1.15 ± 0.05 respectively; $P<0.001$). In conclusion, these data suggest that PGE_2 may regulate neoplastic cell function in an autocrine/paracrine manner via the EP2/EP4 receptors.

Materials and Methods

Tissue collection and Processing. Endometrial adenocarcinoma tissue was collected from women undergoing hysterectomy and who had been pre-diagnosed to have adenocarcinoma of the uterus. All women with endometrial adenocarcinoma were post-menopausal. To provide control

tissue, normal secretory phase (Days 18-25 of the menstrual cycle) endometrial tissue was collected with a pipelle suction curette (Pipelle; Laboratoire CCD, Paris, France) from fertile women with regular menstrual cycles, undergoing gynecological procedures for benign conditions. Biopsies were dated from the patient's last menstrual period (LMP) and histological dating was consistent with date of LMP. Subjects had not been exposed to exogenous hormones for at least six months prior to inclusion in the study. This phase of the menstrual cycle was chosen for comparison with endometrial adenocarcinoma tissue as minimal proliferative activity of the endometrium is detected during the secretory phase (Ferenczy *et al.*, 1979). This would be comparable to the absence of proliferative activity predicted in healthy post-menopausal endometrium. Shortly after hysterectomy or pipelle suction, the tissue was either snap frozen in dry ice and stored at –70°C (for RNA extraction), fixed in Neutral buffered formalin and wax embedded (for immunohistochemical analyses) or placed in RPMI 1640 (containing 2mmol/L L-glutamine, 100 U penicillin and 100 µg/mL streptomycin) and transported to the laboratory for in vitro culture. In addition, archival tissue blocks of healthy post-menopausal uterus were obtained from The Department of Pathology (The University of Edinburgh Medical School) and utilised for immunohistochemical analyses. Written informed consent was obtained prior to tissue collection and ethical approval was received from the Lothian Research Ethics Committee. The data in this study were analysed by ANOVA using StatView 5.0.

Ribonuclease Protection assay. RNA was extracted from endometrial adenocarcinoma tissue (n=3 of each of well, moderately or poorly differentiated endometrial adenocarcinoma) and normal secretory phase endometrium (n=4) using Tri-Reagent as recommended by the manufacturer (Sigma, Dorset, UK). A homologous 381 bp COX-2 cDNA probe was generated by PCR from a clone containing the human COX-2 cDNA (the

clone was a gift from Dr S Prescott, University of Utah) and primers at base pair position 950-971 (COX2A: 5'-CAAGCAGGCTAATACTGATAGG-3') and 1310-1331 (COX2B: 5'-ATCTGCCTGCTCTGGTCAATGG-3'). The amplified PCR product was subcloned into pCRII and its identity and orientation confirmed following sequencing using the Applied Biosystems 373A DNA sequencer and the ABI prism DNA sequencing kit (Applied Biosystems, Cheshire, UK).

For the RPA, an antisense cRNA probe was prepared from HindIII linearised pCRII plasmid containing the 381 bp cDNA fragment of the human COX-2. The RPA was conducted using the Ambion RPA II kit (AMS Biotechnology Europe, Oxfordshire, UK) as previously reported (Jabbour *et al.*, 1998). Briefly, radiolabelled cRNA was generated using the linearised plasmid, T7 RNA polymerase and α^{32}P-UTP (800 Ci/mmol; Amersham, Buckinghamshire, UK). Total RNA (10 μg) from adenocarcinoma tissue and yeast (n=2; used as reaction controls in the presence or absence of RNase digestion to establish the specificity of the hybridisation reaction and the size of the unprotected RNA fragment) was mixed with the radiolabelled probe ($2x10^5$ cpm) and hybridisation buffer, heated to 90°C for 4 min and incubated overnight at 45°C. Integrity of RNA and the relative amount of total RNA in each reaction was determined by including 18S radiolabelled cRNA in each reaction. Single stranded RNA were digested using 250 U/ml RNase A and 10000 U/ml RNase T1 for 30 min at 37°C. The protected RNA was precipitated and separated on a 5% denaturing acrylamide gel. The gel was dried under vacuum and exposed to an autoradiographic film (XAR-5; Kodak).

Immunohistochemistry. Immunohistochemistry was performed on adenocarcinoma tissue (n=4 of each of well, moderately and poorly differentiated), normal secretory phase endometrium (n=4) and healthy post-

menopausal uterus (n=4). Five-micron paraffin wax-embedded tissue sections were dewaxed in xylene, rehydrated in graded ethanol and washed in water followed by TBS (50 mM Tris-HCl, 150 mM NaCl pH 7.4) and blocked for endogenous peroxidase (3% H_2O_2 in methanol). Sections were blocked using either 20% normal rabbit serum (for COX-2), 20% swine serum (for mPGES, PGE_2 and EP4) or 20% normal goat serum (for CD34) diluted in TBS. Subsequently the tissue sections were incubated with polyclonal goat anti-COX-2 antibody (sc-1745; Autogenbioclear, Wilts, UK) at a dilution of 1:400, polyclonal rabbit anti-mPGES antibody (catalogue number 160140; Cayman Chemical, Alexis Corporation-Europe, Nottingham, UK) at a dilution of 1:50, polyclonal rabbit anti-EP4 receptor (catalogue number 101770; Cayman Chemical) at a dilution of 1:500, polyclonal rabbit anti- PGE_2 antibody (kindly supplied by Professor RW Kelly, MRC Human Reproductive Sciences Unit, Edinburgh, UK) at a dilution of 1:100 or monoclonal mouse anti-human CD34 primary antibody (mca-547; Serotec, Oxford, UK) at a dilution of 1:25 at 4°C for 18h. Control tissue was incubated with either 5% non-immune antisera (CD34), goat anti-COX-2 antibody pre-adsorbed to blocking peptide (sc-1745p; Autogenbioclear), rabbit anti-mPGES antibody pre-adsorbed to blocking peptide (catalogue number 360140; Cayman Chemical), rabbit anti-EP4 pre-adsorbed to blocking peptide (catalogue number 101780; Caman Chemical) or rabbit anti-PGE_2 antibody pre-adsorbed to 10-fold excess PGE_2 (Sigma). After thorough washing with TBS, the tissue sections probed with the goat anti-human COX-2, rabbit anti-mPGES, rabbit anti-EP4 and rabbit anti-PGE_2 primary antibodies were incubated with biotinylated rabbit anti-goat secondary IgG antibody (for COX-2; Dako, Bucks, UK) or swine anti-rabbit secondary IgG antibody (for mPGES, EP4 and PGE_2; Dako) at a dilution of 1:500 for 40 min at 25°C. Thereafter the tissue sections were incubated with streptavidin-biotin peroxidase complex (Dako) for 20 min at 25°C. Tissue sections probed with the mouse anti-

human CD34 antibody were developed using a Mouse EnVision Kit (Dako) as instructed by the manufacturer. Colour reaction was developed by incubation with 3.3'-diaminobenzidine (Dako).

Real time quantitative PCR. Endometrial RNA samples were extracted from adenocarcinoma tissue (n=4 well differentiated, n=6 moderately differentiated, n=4 poorly differentiated) and normal secretory phase endometrium (n=7) as described above. RNA samples were reverse transcribed using $MgCl_2$ (5.5 mM), dNTPs (0.5 mM each), random hexamers (2.5 μM), RNAase inhibitor (0.4 U/μl) and multiscribe reverse transcriptase (1.25 U/μl; all from PE Biosystems, Warrington, UK). The mix was aliquoted into individual tubes (16 μl/tube) and template RNA was added (4 μl/tube of 100 ng/μl RNA). Samples were incubated for 60 minutes at 25°C, 45 minutes at 48°C and then at 95°C for 5 minutes.

A reaction mix was made containing Taqman buffer (5.5 mM $MgCl_2$, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 400 μM dUTP), ribosomal 18S forward and reverse primers and probe (all at 50 nM), forward and reverse primers for EP receptor (300 nM), EP receptor probe (200 nM), AmpErase UNG (0.01 U/μl) and AmpliTaq Gold DNA Polymerase (0.025 U/μl; all from PE Biosystems). A volume of 48 μl of reaction mix was aliquoted into separate tubes for each cDNA sample and 2 μl/replicate of cDNA was added. After mixing, 23 μl of sample were added to the wells on a PCR plate. Each sample was added in duplicate. A no template control (containing water) was included in triplicate. Wells were sealed with optical caps and the PCR reaction run on an ABI Prism 7700 using standard conditions. EP receptor primers and probe for quantitative PCR were designed using the PRIMER express program (PE Biosystems). The sequence of the EP2 receptor primers and probe were as follows; Forward: 5'-GAC CGC TTA CCT GCA GCT GTA C-3'; Reverse: 5'–TGA AGT

TGC AGG CGA GCA-3'; Probe (FAM labelled): 5'–CCA CCC TGC TGC TGC TTC TCA TTG TCT-3'. The sequence of the EP4 receptor primers and probe were as follows; Forward: 5'–ACG CCG CCT ACT CCT ACA TG-3'; Reverse: 5'–AGA GGA CGG TGG CGA GAA T-3'; Probe (FAM labelled): 5'–ACG CGG GCT TCA GCT CCT TCC T-3'. The ribosomal 18S primers and probe sequences were as follows; Forward: 5' –CGG CTA CCA CAT CCA AGG AA-3'; Reverse: 5'–GCT GGA ATT ACC GCG GCT-3'; Probe (VIC labelled): 5'–TGC TGG CAC CAG ACT TGC CCT C-3'.

In vitro culture and cAMP measurement. Endometrial tumour tissue (n=6; two well, two moderately and two poorly differentiated adenocarcinomas) and normal secretory phase endometrium (n=6) were minced finely with scissors and incubated in 2 ml RPMI (Sigma) medium containing 10% fetal calf serum, 0.3 mg/ml L-glutamine, 100 IU penicillin, 100 μg streptomycin and 3 μg/ml indomethacin, for 1.5 hrs at 37C in humidified 5% CO_2. Thereafter samples were incubated in the same medium containing IBMX (Sigma) to a final concentration of 1 mM for 30 min at 37C and then stimulated for 5 min with 300 nM PGE_2. Control treatments received no PGE_2. Tissue was harvested by centrifugation at 2000g. The supernatant was discarded and the tissue homogenised in 0.1 M HCl. cAMP concentration was quantified by ELISA using a cAMP kit (Biomol; Affiniti, Exeter, UK) and normalised to protein concentration of the homogenate. Protein concentrations were determined using protein assay kits (Bio-Rad, Hemel Hempstead, UK). The data are presented as fold induction of cAMP following treatment with PGE_2. Fold induction was calculated by dividing cAMP values for the PGE_2-treated samples by the value for the untreated sample.

Results

COX-2 expression in endometrial adenocarcinoma was assessed by ribonuclease protection assay. COX-2 expression was detected in well, moderately and poorly differentiated (n=3 each grade) adenocarcinomas (Figure 1). No COX-2 expression was detected in the secretory phase endometrium collected from women with normal menstrual cycles (Figure 1). It was not possible to obtain fresh healthy post-menopausal endometrium to assess COX-2 RNA expression and hence secretory phase endometrium was chosen as a comparative tissue for the adenocarcinoma. This phase of the menstrual cycle was chosen as minimal proliferative activity is detected in the endometrium during the secretory phase (Ferenczy *et al.*, 1979). This would be comparable to the absence of proliferative activity predicted in healthy post-menopausal endometrium.

Immunohistochemistry was employed in order to detect the site of expression of COX-2/mPGES and synthesis of PGE_2 in the endometrial adenocarcinomas. COX-2 and mPGES expression was detected in neoplastic epithelial cells in poorly, moderately and well differentiated adenocarcinoma (Figure 2a, 2c and 2e for COX-2 and Figure 3a, 3b and 3c for mPGES respectively). Minimal signal was detected in archival post-menopausal uterine specimens or secretory phase endometrium (Figures 2g, 2i, 3d and 3e) and no signal was detected when the antibody was pre-adsorbed with blocking peptide (Figures 2e and 3c insets). Similar to COX-2/mPGES, PGE_2 synthesis was detected in the neoplastic epithelial cells of carcinomas of different grades of differentiation (Figure 2b, 2d and 2f respectively). Minimal immunostaining for PGE_2 was observed in post-menopausal uterus or secretory phase endometrium (Figure 2h and 2j respectively) and no staining was detected in tissue treated with pre-adsorbed sera (Figure 2f inset). In addition, COX-2, mPGES and PGE_2 immunostaining was observed in endothelial cells lining the vasculature in

all adenocarcinoma sections investigated (Figure 4a, 4b and 4c respectively). To confirm that COX-2/mPGES expression and PGE_2 synthesis were localised to the endothelial cells of blood vessels, immunohistochemistry was performed on tissue sections using antibodies raised against the CD34 endothelial cell marker. The pattern of expression with CD34 (Figure 4e) was identical to that observed with COX-2, mPGES and PGE_2 thus confirming that COX-2/mPGES expression and PGE_2 synthesis are localised to the endothelial cell layer of blood vessels in human adenocarcinomas. Negligible staining was observed in the stromal compartment of all carcinoma tissue investigated.

The expression of two subtypes of PGE_2 receptors, namely EP2 and EP4, was investigated by real-time quantitative PCR in carcinoma tissue and normal secretory phase endometrium. Expression of both receptors was significantly up-regulated in adenocarcinoma tissues compared with normal secretory endometrium ($P<0.01$). No differences in the level of expression of EP2 or EP4 receptors were detected between poorly, moderately or well differentiated adenocarcinomas (Figure 5A). Overall, the fold induction of EP2 and EP4 receptor expression in adenocarcinoma tissue (mean fold induction of all carcinoma samples) compared with normal secretory endometrium was 28.0 ± 7.4 and 52.5 ± 10.1 for EP2 and EP4 receptors respectively. Using immunohistochemistry, EP4 receptor expression was localised to neoplastic epithelial cells of carcinoma tissues of all grades of differentiation (Figure 5B) and also in endothelial cells of the microvasculature (Figure 4d).

In order to assess the activity of the EP2/EP4 receptors in the carcinoma tissue and normal secretory phase endometrium, cAMP generation was measured following short term in vitro culture with or without PGE_2 (Figure 6). Comparable cAMP turnover in response to PGE_2 was observed in all

carcinoma tissue. The fold induction of cAMP generation in response to PGE_2 was significantly higher in carcinoma tissue compared with secretory phase endometrium (3.42 ± 0.46 vs 1.15 ± 0.05 respectively; $P<0.001$).

Discussion

The data presented in this study demonstrate the expression of COX-2 and mPGES enzymes in adenocarcinomas of the uterus at different grades of differentiation as demonstrated by ribonuclease protection assays and immunohistochemistry. COX-2 and mPGES expression were co-localised to neoplastic epithelial cells and endothelial cells of the microvasculature suggesting a co-regulated pattern of expression for the two genes. Previous studies have detected expression of mPGES in human smooth muscle vascular cells but not in umbilical vein endothelial cells although these cells retained synthetic capacity for PGE_2 (Soler *et al.*, 2000). This apparent discrepancy may reflect tissue variation in regulation of expression of mPGES in endothelial cells. The over-expression of COX-2 enzyme observed in endometrial adenocarcinomas resembles that reported for a number of other carcinomas including colon, lung, bladder, stomach, pancreas, prostate and cervix (Gupta *et al.*, 2000; Mohammed *et al.*, 1999; Ratnasinghe *et al.*, 1999; Sales *et al.*, 2001; Tsujii *et al.*, 1997; Tucker *et al.*, 1999; Wolff *et al.*, 1998). The exact intracellular signalling pathways that lead to up-regulation in COX-2 expression in carcinomas remain to be elucidated. However, recent data suggest regulatory roles for ERK2 MAP kinase (Jones *et al.*, 1999a), p38 MAP kinase (Dean *et al.*, 1999) and Phosphatidylinositol 3-kinase (Weaver *et al.*, 2001) in a number of model systems including endometrial adenocarcinoma epithelial cells (Munir *et al.*, 2000).

The immunohistochemistry studies suggest that COX-2 and mPGES expression is associated with enhanced production of PGE_2 in neoplastic

cells and endothelial cells of the microvasculature. Previous studies have shown that PGE_2 synthesis/secretion is significantly elevated in uterine carcinomas compared with normal uterus (Willman *et al.*, 1976). Moreover, in a number of model systems PGE_2 synthesis and secretion are elevated in response to COX-2 up-regulation (Tsujii & DuBois, 1995). The biological role of COX-2 and PGE_2 in endometrial adenocarcinomas remains to be established. However, enhanced COX-2 expression and PGE_2 synthesis can induce neoplastic changes in epithelial cells through a number of biological pathways. These include promotion of cellular proliferation, inhibition of apoptosis, increasing metastatic potential of neoplastic cells and promoting angiogenesis (Rolland *et al.*, 1980; Tsuji *et al.*, 1996; Tsujii & DuBois, 1995). Over-expression of COX-2 enzyme in rat intestinal epithelial cells results in enhanced secretion of PGE_2 which is associated with increased cellular proliferation and resistance to apoptosis (Tsujii & DuBois, 1995). Similarly, over expression of COX-2 and increased production of prostaglandins have been linked to enhanced metastatic potential of neoplastic cells possibly through down regulation of expression of cell adhesion molecules such as E-Cadherin (Rolland *et al.*, 1980). Expression of E-Cadherin is down regulated in a number of solid tumours and is closely and inversely related to enhanced invasion of neoplastically transformed cells (Mayer *et al.*, 1993; Schipper *et al.*, 1991).

Successful tumour establishment and metastasis is also dependent on initiation of angiogenesis at the site of growth of the tumour cells. COX-2 and PGE_2 are strongly linked with regulation of the angiogenic process during tumour development (Masferrer *et al.*, 2000). Over-expression of COX-2 and increased production of PGE_2 in epithelial cells enhances the expression of angiogenic factors which act in a paracrine manner to induce endothelial cell migration and microvascular tube formation (Tsujii *et al.*, 1998). Similarly, COX-2 and PGE_2 may influence angiogenesis directly by

acting on endothelial cells. Treatment of endothelial cells with selective COX-2 inhibitors has been shown to reduce microvascular tube formation and this effect is partially reversed by co-treatment with PGE_2 (Jones *et al.*, 1999b). Hence, it is feasible that in vivo angiogenesis in endometrial adenocarcinomas may be regulated by COX-2 and PGE_2 via an epithelial-endothelial and an endothelial-endothelial cell interaction. This is supported by the data presented in this study which localised the site of expression of COX-2, mPGES and PGE_2 to neoplastic epithelial cells and endothelial cells.

PGE_2 acts on target cells through interaction with seven transmembrane G-protein coupled receptors. Different forms of the membrane bound receptors have been cloned which utilise alternate intracellular signalling pathways. In this study we investigated the expression of two of the membrane bound PGE_2 receptors, namely EP2 and EP4, which mediate their effect on target cells via the PKA pathway by activating adenylate cyclase and increasing intracellular cAMP (Coleman *et al.*, 1994). In endometrial adenocarcinoma, expression of EP2 and EP4 receptors is up-regulated in comparison with normal secretory phase endometrium and expression of at least the EP4 receptor is localised to neoplastic epithelial cells and the endothelium of the microvasculature. It was not possible to conduct parallel studies to localise EP2 receptors in the carcinoma tissues as no commercial antibodies are available for this receptor. Hence it remains to be established whether these receptors are co-expressed in the same cell type. However, using in situ hybridisation techniques, EP2 and EP4 receptor expression have been recently co-localised to epithelial and endothelial cells of the normal human endometrium (Milne *et al.*, 2001). Functionality of the EP2/EP4 receptors in the carcinoma tissue was assessed by measuring cAMP generation following treatment with exogenous PGE_2. Treatment with PGE_2 resulted in a rapid cAMP generation thus demonstrating

functional activation of the EP2 and/or EP4 receptors in this tissue. Up-regulation in expression and signalling of EP2/EP4 receptors has also been reported in cervical carcinoma and this suggests a common signalling pathway for PGE_2 in reproductive tract neoplasia (Sales *et al.*, 2001). The exact role of COX-2/mPGES enzymes and PGE_2 and the associated EP2/EP4 receptors in endometrial adenocarcinoma remains to be established. However, it is reasonable to suggest that COX-2/mPGES and PGE_2 may mediate proliferation of epithelial and/or endothelial cells. The proliferating cells within endometrial adenocarcinomas are detected predominantly in post-menopausal women at a time when the healthy endometrium is expected to atrophy and display minimal cellular proliferation or angiogenesis. Moreover, healthy post-menopausal uterus and normal secretory endometrium, both of which have minimal proliferative or angiogenic activity, display negligible COX-2/mPGES/EP2/EP4 receptor expression and minimal cAMP generation in response to treatment with PGE_2. A possible role for PGE_2 in proliferation has already been established in a number of cell types including endothelial cells and it has been suggested that this effect is mediated via cAMP and induction of expression of mitogenic growth factors such as vascular endothelial growth factor and basic fibroblast growth factor (Cheng *et al.*, 1998; Hoper *et al.*, 1997). Future studies will elucidate the exact role of PGE_2 and its associated receptors on proliferation and neoplastic differentiation of epithelial/endothelial cells in endometrial adenocarcinomas.

In conclusion, these data confirm the expression of COX-2 and mPGES enzymes and synthesis of PGE_2 in endometrial adenocarcinoma of various grades of differentiation. Both COX-2/mPGES and PGE_2 are localised to the neoplastic epithelial cells and endothelial cells of the microvasculature. PGE_2 may exert an autocrine/paracrine effect in endometrial

adenocarcinoma through interaction with EP2/EP4 receptors and activation of the PKA signalling pathway.

Example 2: Treatment of uterine cancer with an EP2 receptor antagonist

A patient suffering from uterine cancer is administered AH6809. Optionally, the patient is also administered meloxicam.

Example 3: Treatment of fibroids with EP4 receptor antagonist

A patient suffering from fibroids is administered AH23848B.

Example 4: Treatment of endometriosis with an EP4 receptor antagonist

A patient suffering from endometriosis is administered AH22921X. Optionally, the patient is also administered nimesulide.

References for Example 1

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CLAIMS

1. A method of treating or preventing a pathological condition of the uterus in an individual the method comprising administering to the individual any one or more of an inhibitor of prostaglandin E synthase (PGES) or an EP2 or EP4 receptor antagonist.

2. A method according to Claim 1 wherein the pathological condition of the uterus is associated with abnormal growth of cells of the myometrium or endometrium.

3. A method according to Claim 1 or 2 wherein the pathological condition of the uterus is uterine carcinoma or an endometrial or myometrial pathological condition.

4. A method according to Claim 3 wherein the endometrial pathological condition is endometriosis.

5. A method according to Claim 3 wherein the myometrial pathological condition is fibroids.

6. A method according to any one of Claims 1 to 5 wherein a PGE synthase inhibitor is administered to the individual.

7. A method according to any one of Claims 1 to 6 wherein an EP2 receptor antagonist or EP4 receptor antagonist is administered to the individual.

8. A method according to Claim 7 wherein the individual is administered any one or more of AH6809, an omega-substituted

prostaglandin E derivative described in WO 00/15608 (Ono Pharm Co Ltd), AH23848B, AH22921X, IFTSYLECL, IFASYECL, IFTSAECL, IFTSYEAL, ILASYECL, IFTSTDCL, TSYEAL (with 4-biphenylalanine), TSYEAL (with homophenylalanine), a 5-thia-prostaglandin E derivative described in WO 00/03980 (Ono Pharm Co Ltd), 5-butyl-2,4-dihydro-4-[[2'-[N-(3-chloro-2-thiophenecarbonyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one potassium salt, 5-butyl-2,4-dihydro-4-[[2'-[N-(2-methyl-3-furoyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one, 5-butyl-2,4-dihydro-4-[[2'-[N-(3-methyl-2-thiophenecarbonyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one, 5-butyl-2,4-dihydro-4-[[2'-[N-(2-thiophenecarbonyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one, and 5-butyl-2,4-dihydro-4-[[2'-[N-[2-(methypyrrole)carbonyl]sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one.

9. A method according to any one of Claims 1 to 8 wherein an EP2 receptor antagonist is administered to the individual.

10. A method according to Claim 9 wherein the EP2 receptor antagonist is AH6809.

11. A method according to any one of Claims 1 to 10 wherein an EP4 receptor antagonist is administered to the individual.

12. A method according to Claim 11 wherein the EP4 receptor antagonist is any one or more of AH23848B, AH22921X, IFTSYLECL, IFASYECL, IFTSAECL, IFTSYEAL, ILASYECL,

IFTSTDCL, TSYEAL (with 4-biphenylalanine), TSYEAL (with homophenylalanine), and 5-thia-prostaglandin E derivatives described in WO 00/03980 (Ono Pharm Co Ltd), 5-butyl-2,4-dihydro-4-[[2'-[N-(3-chloro-2-thiophenecarbonyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one potassium salt, 5-butyl-2,4-dihydro-4-[[2'-[N-(2-methyl-3-furoyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one, 5-butyl-2,4-dihydro-4-[[2'-[N-(3-methyl-2-thiophenecarbonyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one, 5-butyl-2,4-dihydro-4-[[2'-[N-(2-thiophenecarbonyl)sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one, and 5-butyl-2,4-dihydro-4-[[2'-[N-[2-(methypyrrole)carbonyl]sulfamoyl]biphenyl-4-yl]methyl]-2-{2-(trifluoromethyl)phenyl]-1,2,4-triazol-3-one.

13. A method according to any of Claims 1 to 12 further comprising administering a COX-2 inhibitor to the individual.

14. A method according to Claim 13 wherein the COX-2 inhibitor is any one of any one of nimesulide, 4-hydroxynimesulide, flosulide, and meloxicam.

15. Use of any one or more of an inhibitor of prostaglandin E synthase (PGES), or an EP2 or EP4 receptor antagonist in the manufacture of a medicament for treating or preventing a pathological condition of the uterus.

16. Use according to Claim 15 wherein the pathological condition of the uterus is uterine carcinoma or an endometrial or myometrial pathological condition.

17. Use according to Claim 15 or 16 wherein the medicament is for administration to a patient who is administered a COX-2 inhibitor and/or an inhibitor of PGES and/or an EP2 or EP4 receptor antagonist.

18. Use according to Claim 15 or 16 wherein the medicament further comprises an inhibitor of COX-2.

Figure 1

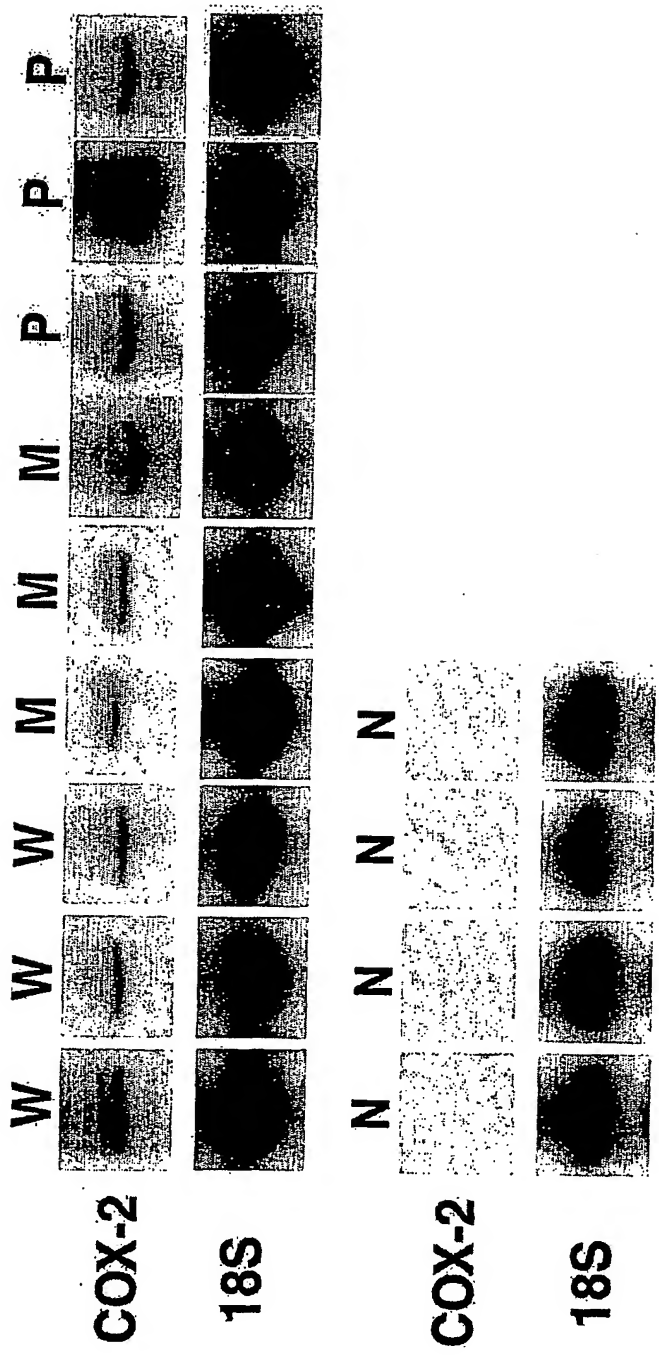


Figure 2

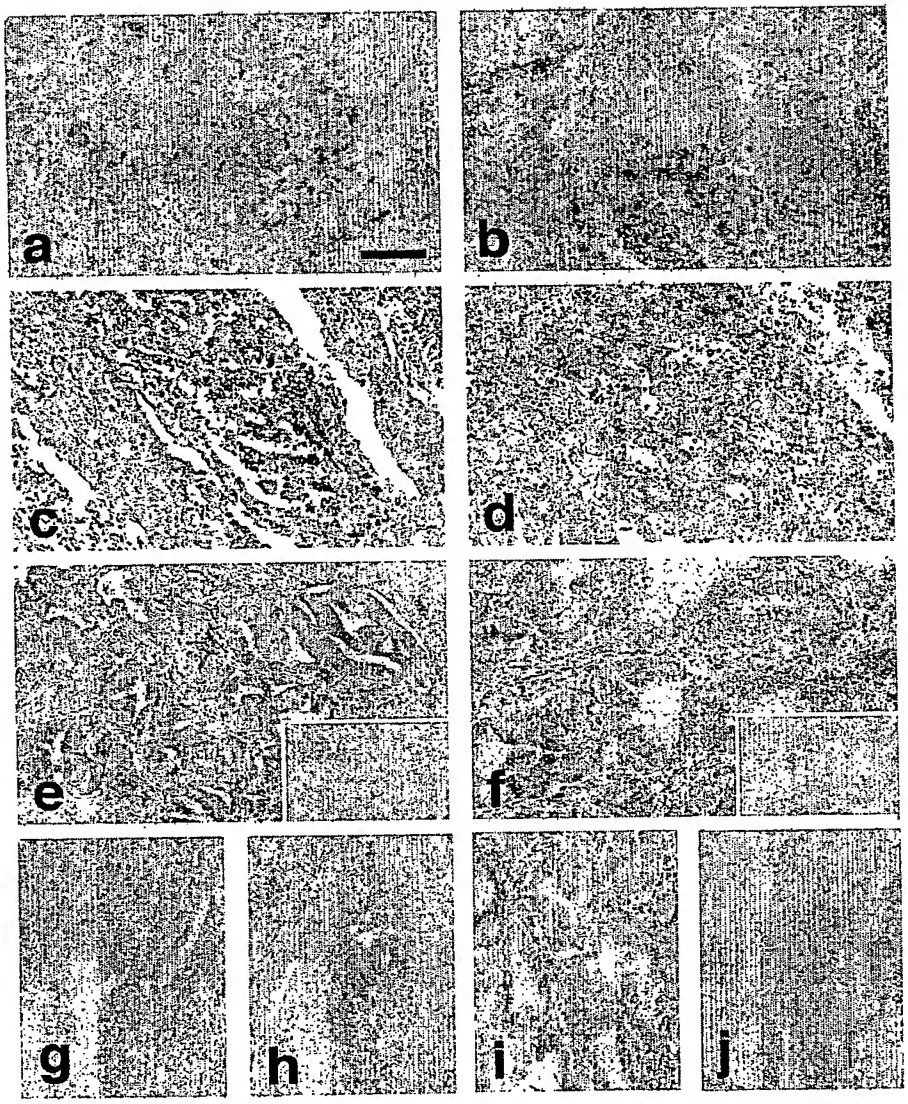


Figure 3

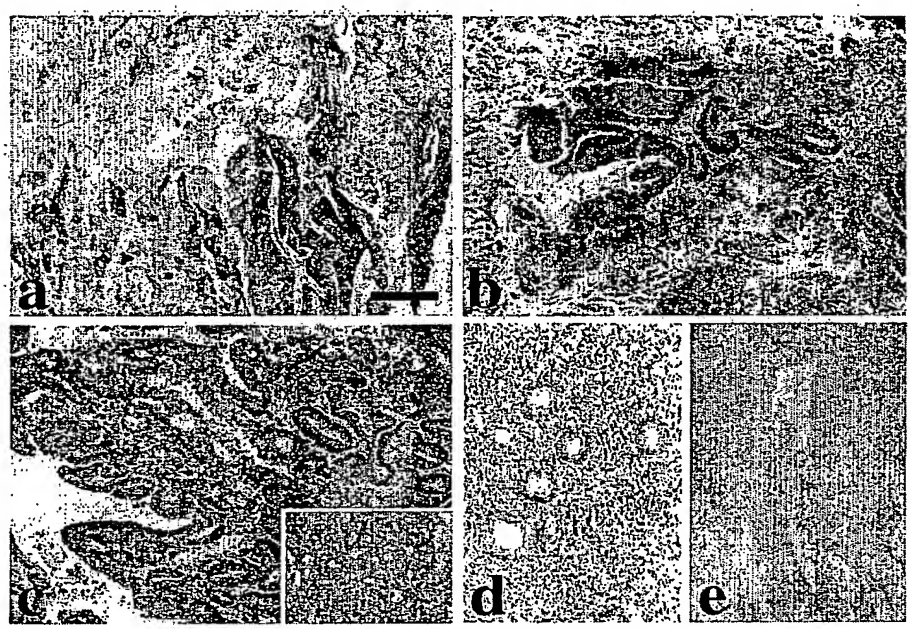


Figure 4

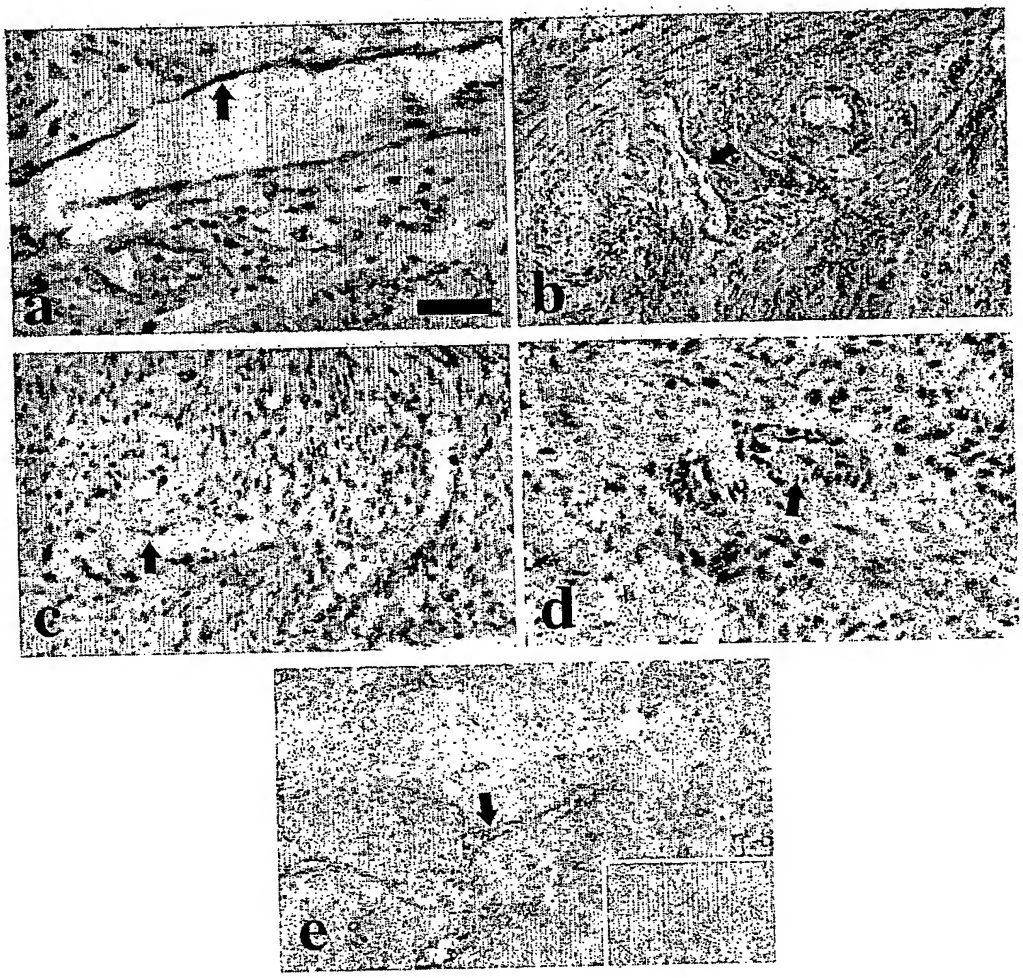
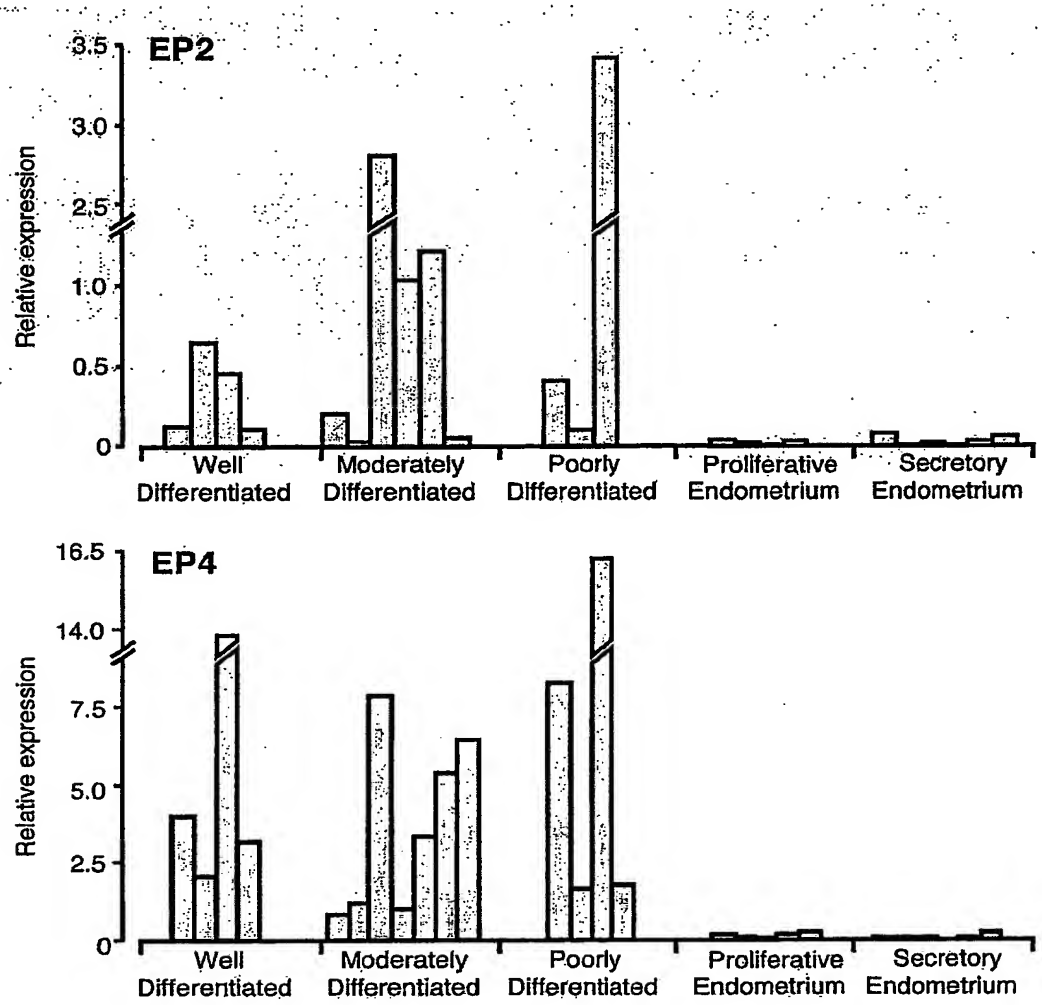


Figure 5: page 1



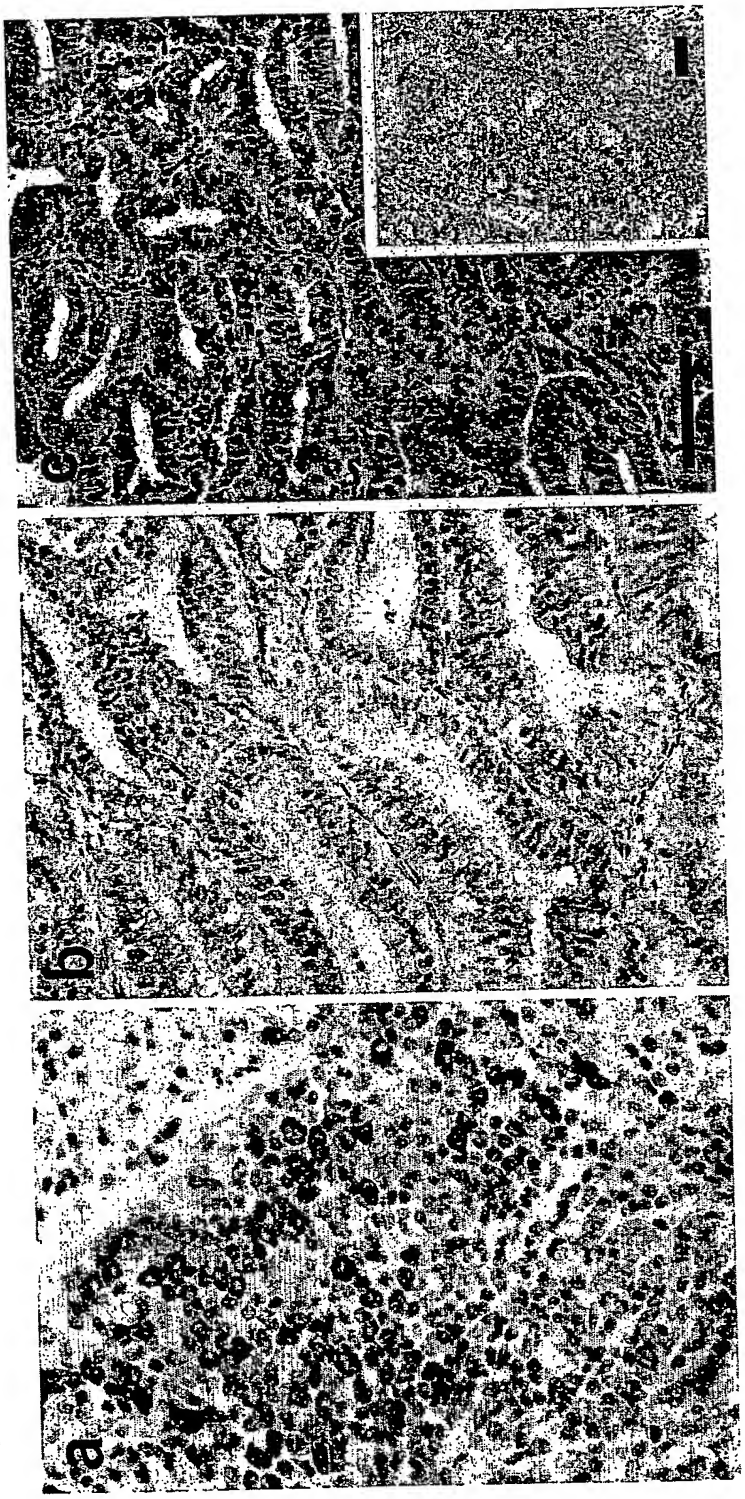
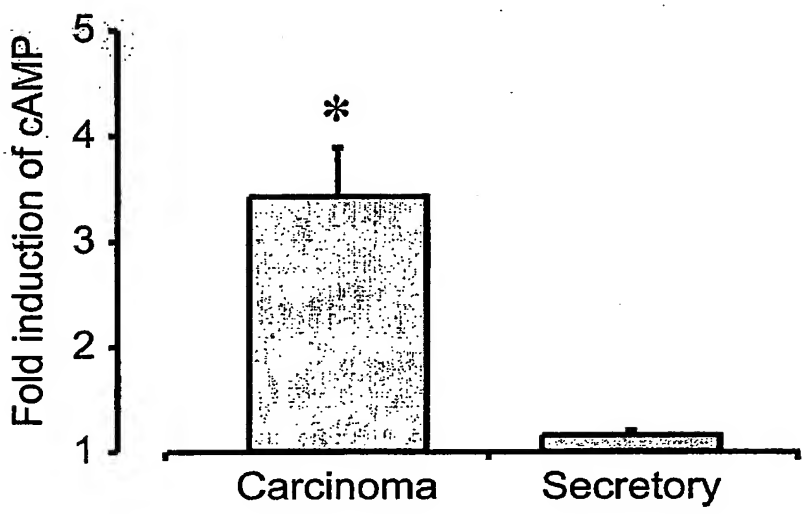


Figure 5: page 2

Figure 6



INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/GB 02/04549

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/557 A61K38/08 A61K31/41 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 1 114 816 A (ONO PHARMACEUTICAL CO) 11 July 2001 (2001-07-11) cited in the application the whole document	8,12
A	WO 01 42281 A (CHEMTOB SYLVAIN ;PERI KRISHNA G (CA); HOPITAL SAINTE JUSTINE (CA)) 14 June 2001 (2001-06-14) cited in the application the whole document	8,12
A	EP 1 097 922 A (ONO PHARMACEUTICAL CO) 9 May 2001 (2001-05-09) cited in the application the whole document	8,12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 February 2003	27/02/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Deck, A

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INTERNATIONAL SEARCH REPORT

Internatk Application No
PCT/GB 02/04549

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 21532 A (MERCK FROSST CANADA INC ;LABELLE MARC (CA); YOUNG ROBERT N (CA); M) 20 April 2000 (2000-04-20) cited in the application the whole document	8,12
A	JABBOUR H N ET AL: "Expression of COX-2 and PGE synthase and synthesis of PGE2 in endometrial adenocarcinoma: A possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors." BRITISH JOURNAL OF CANCER, vol. 85, no. 7, 28 September 2001 (2001-09-28), pages 1023-1031, XP009005407 ISSN: 0007-0920 the whole document	1-18
A	SALES K J ET AL: "Cyclooxygenase-2 expression and prostaglandin E2 synthesis are up-regulated in carcinomas of the cervix: A possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors." JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM, vol. 86, no. 5, May 2001 (2001-05), pages 2243-2249, XP002230684 ISSN: 0021-972X cited in the application the whole document	13,14, 17,18
A	MASFERRER JAIME L ET AL: "Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors." CANCER RESEARCH, vol. 60, no. 5, 1 March 2000 (2000-03-01), pages 1306-1311, XP002230685 ISSN: 0008-5472 cited in the application the whole document	13,14, 17,18
A	HILLOCK C J ET AL: "INHIBITORY PROSTANOID EP RECEPTORS IN HUMAN NON-PREGNANT MYOMETRIUM" EUROPEAN JOURNAL OF PHARMACOLOGY, AMSTERDAM, NL, vol. 378, no. 1, 28 July 1999 (1999-07-28), pages 99-108, XP001124311 ISSN: 0014-2999 cited in the application the whole document	8,12

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INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/GB 02/04549

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PELLETIER S ET AL: "PROSTAGLANDIN E2 INCREASES CYCLIC AMP AND INHIBITS ENDOTHELIN-1 PRODUCTION/SECRETION BY GUINEA-PIG TRACHEAL EPITHELIAL CELLS THROUGH EP4 RECEPTORS" BRITISH JOURNAL OF PHARMACOLOGY, BASINGSTOKE, HANTS, GB, vol. 132, no. 5, March 2001 (2001-03), pages 999-1008, XP009004865 ISSN: 0007-1188 cited in the application the whole document	8,12

INTERNATIONAL SEARCH REPORT

Inte, ɔnal application No.
PCT/GB 02/04549

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat Application No
PCT/GB 02/04549

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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